

# EXHIBIT B

## (Part 3 of 3)

WO 93/17126

PCT/US93/01552

-80-

173. A method according to claim 172 wherein the substantially complete set of partials is prepared by chemical or enzymatic degradation of the strands and the strands are sorted on a binary oligonucleotide array, said binary array comprising an array or predetermined areas on a surface of a solid support, each area having therein copies of an binary oligonucleotides of a predetermined sequence, said binary oligonucleotide consisting of a constant nucleotide sequence of predetermined length and nucleotide sequence adjacent to a variable nucleotide sequence.

174. A method according to claim 173 wherein said binary oligonucleotide array comprises a 3' array, said immobilized oligonucleotides consisting of a constant sequence at the 5' terminus of a variable sequence.

175. A method according to claim 172 further comprising

(a) preparing address sets containing a complete list of all oligonucleotides contained in a strand or strands in the mixture which share an address oligonucleotide for substantially every address in the oligonucleotide array on which the partials were sorted; and

(b) determining whether an address set is a strand set by examining whether the address set can be decomposed into other address sets.

176. A method according to claim 175 further comprising organizing the oligonucleotides in a strand set into sequence blocks composed of oligonucleotides that uniquely overlap each other, and ordering the blocks.

177. A method of obtaining information to order a set of first fragments resulting from digestion of DNA with a first restriction endonuclease, the nucleotide sequence of said fragments having already been determined, comprising

(a) digesting the DNA with a second restriction endonuclease to generate a set of second fragments;

WO 93/17126

PCT/US93/01552

-81-

(b) denaturing the second set of fragments to form a mixture of single nucleic acid strands;

(c) sorting strands on a substantially comprehensive oligonucleotide array;

(d) amplifying the strands to generate both their direct and complementary copies;

(e) surveying the contents of individual areas of the array on a first binary survey array, said first binary survey array comprising an array of predetermined areas on a surface of a solid support, each area having therein, covalently linked to said surface, copies of a binary oligonucleotide, said binary oligonucleotide having a constant nucleotide sequence which contains a sequence complementary to the restriction recognition site of the first restriction endonuclease and adjacent to a variable sequence; and

(f) surveying the contents of individual areas of the array on a second binary survey array, said second binary survey array comprising an array of predetermined areas on a surface of a solid support, each area having therein, covalently linked to said surface, copies of a second binary oligonucleotide, said second binary oligonucleotide having a constant nucleotide sequence which contains a sequence complementary to the restriction recognition site of the second restriction endonuclease and adjacent to a variable sequence.

178. A method according to claim 177 wherein in step c strands are hybridized to an array selected from the group consisting of

(a) a first binary sorting array, said first binary sorting array comprising an array of immobilized oligonucleotides having a constant nucleotide sequence complementary to the restriction recognition site of the first restriction endonuclease, adjacent to a variable sequence of predetermined length, the immobilized oligonucleotides in an individual area of the first binary sorting array having the same sequence, and

(b) a second binary sorting array, said second binary sorting array comprising an array of immobilized oligonucleotides having a constant nucleotide sequence complementary to the

WO 93/17126

PCT/US93/01552

-82-

restriction recognition site of the second restriction endonuclease, adjacent to a variable sequence of predetermined length, the immobilized oligonucleotides in an individual area of the second binary sorting array having the same sequence, and wherein following hybridization unhybridized and imperfectly hybridized strands are removed.

179. A method for obtaining information to allocate sequenced and ordered fragments from an original restriction digest of DNA from sister chromosomes to chromosomal linkage groups comprising

(a) preparing a partial on an oligonucleotide array from a restriction fragment from an alternate restriction digest of the DNA, which partial spans first and second allelic differences in neighboring pairs of sequenced fragments from the original restriction digest; and

(b) determining the presence of oligonucleotides containing the first and second allelic differences in a partial which spans the first and second allelic differences.

180. A method according to claim 179 wherein

(a) in step b, the restriction fragment from the alternate restriction digest is hybridized to the oligonucleotide array by an oligonucleotide containing the first allelic difference; and

(b) the presence of an oligonucleotide containing the second allelic difference is determined by hybridizing the partial to a complementary second variable nucleotide sequence in an oligonucleotide array and then detecting the presence of the partial in the corresponding area of the oligonucleotide array.

181. A method for surveying oligonucleotides in a nucleic acid strand comprising

(a) randomly degrading the strand into pieces, the average length of said pieces slightly exceeding the length of oligonucleotides surveyed;

(b) ligating the pieces to a ligating oligonucleotide complementary to at least a portion of a constant sequence of immobilized oligonucleotides in a binary array;

WO 93/17126

PCT/US93/01552

-83-

- (c) hybridizing the pieces to the binary array, said binary array having immobilized oligonucleotides in an ordered array therein and consisting of a constant sequence adjacent to a variable sequence, the immobilized oligonucleotides in an individual area of the array having the same sequence; and
- (d) detecting the hybrids formed.

182. A method according to claim 181 wherein the array is a 3' array having the variable sequence at the 3' termini of the immobilized oligonucleotides, further comprising, following step (c), extending the immobilized oligonucleotides with a polymerase using hybridized pieces as templates.

183. A method according to claim 182 wherein the strand is a DNA strand resulting from a digest with a restriction endonuclease, and melting apart of the fragments obtained thereby or a partial obtained from said strand, and wherein the constant sequence contains the restriction endonuclease recognition site.

184. A method according to claim 183 wherein dideoxynucleotides are used as substrates during extension of the immobilized oligonucleotides using a DNA polymerase.

185. A method according to claim 181 wherein the ligating oligonucleotide is pre-hybridized to the constant immobilized oligonucleotide prior to ligation to the pieces.

186. In a primer dependent polymerase reaction for amplification of a nucleic acid in which a primer is hybridized to a template strand and extended by incubation with a primer dependent polymerase and nucleotide substrates to generate a complementary copy of the template strand; the improvement wherein:

the primer or a part thereof contains one or more primer nucleotides that are chemically different from nucleotide substrates incorporated in the complementary copy of the template during the amplification said chemical difference causing the

WO 93/17126

PCT/US93/01552

-84-

primer to be cleavable without cleaving the part of the complementary copy generated during amplification.

187. A method according to claim 186 further wherein the primer is selectively cleaved without cleaving the part of the complementary copy generated during amplification.

188. A method according to 187 wherein the primer or a part thereof contains one or more ribonucleotides triphosphates, and the substrates used for amplification are deoxyribonucleoside triphosphates and the primer is cleaved by a chemical or enzymatic reaction which cleaves nucleic strands immediately 3' of ribonucleotides but not 3' of deoxyribonucleotides.

189. A method according to claim 188 wherein the chemical reaction or enzymatic reaction is selected from the group consisting of

- (a) alkaline hydrolysis;
- (b) hydrolysis by a magnesium formamide mixture; and
- (c) ribonuclease digestion.

190. A method according to claim 188 wherein a ribonucleotide is present at the 3' terminus of the primer.

191. A method according to claim 187 wherein said nucleotide substrates used for amplification are modified at their alpha phosphate groups so that resulting modified phosphodiester bonds in the complementary copy generated during amplification is resistant to cleavage by a nuclease, said nuclease being chosen to be incapable of cleaving said resulting modified phosphodiester bonds, further wherein one or more primer phosphodiester bonds are not modified to be resistant to said cleavage, and wherein said primer is cleaved by treatment with said nuclease.

WO 93/17126

PCT/US93/01552

-85-

192. A method according to claim 191 wherein said nucleotide substrates modified at their alpha phosphate groups are nucleoside alpha-thiophosphates.

193. A method according to claim 191 wherein the nucleotide substrates used for amplification are modified deoxy-ribonucleotides.

194. An array of oligonucleotide arrays comprising a solid sheet having a surface and an array comprising a pattern of miniaturized oligonucleotide arrays on said surface, each miniaturized array comprising an array of predetermined areas on said surface, each area having therein, covalently linked to said surface, multiple copies of an oligonucleotide of a predetermined sequence.

195. A method according to claim 68 further comprising

(a) contacting at least one area of said array containing the immobilized copies with at least one oligonucleotide probe having a predetermined sequence, under conditions promoting hybridization of said at least one probe; and

(b) determining whether or not said at least one probe has hybridized to said at least one area.

196. A method according to claim 144 further comprising

(a) contacting at least one area of said array containing the immobilized partial copies with at least one oligonucleotide probe having a predetermined sequence, under conditions promoting hybridization of said at least one probe; and

(b) determining whether or not said at least one probe has hybridized to said at least one area.

197. A method according to claim 170, wherein determining the presence and sequence of all variable oligonucleotides comprises

(a) contacting said substantially complete set of partials with a substantially comprehensive set of oligonucleotide probes;

WO 93/17126

PCT/US93/01552

-86-

each of a predetermined length, under conditions promoting hybridization of said probes; and

(b) determining to which partials each said probe has hybridized.

WO 93/17126

1/14

PCT/US93/01552

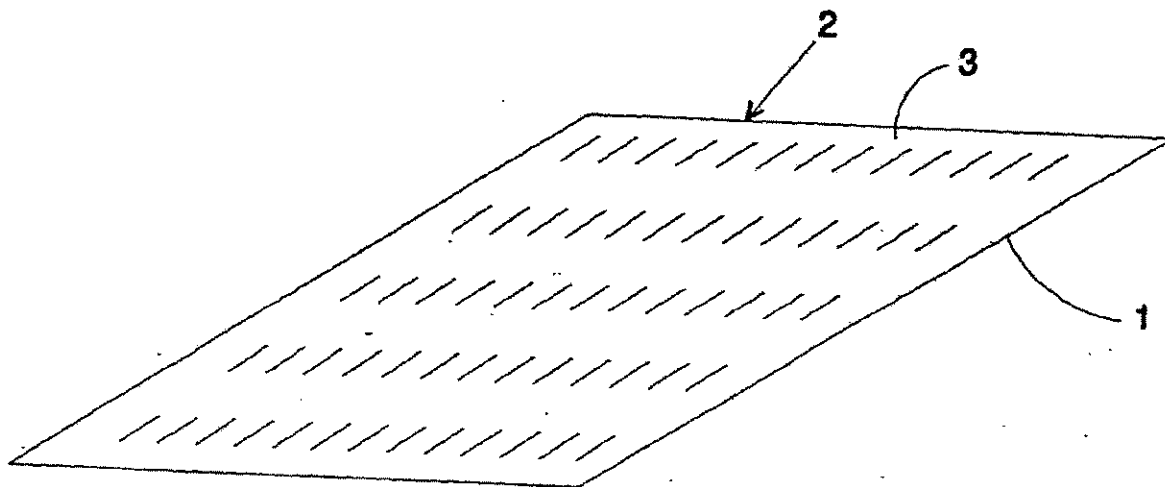


Figure 1 - Binary Array

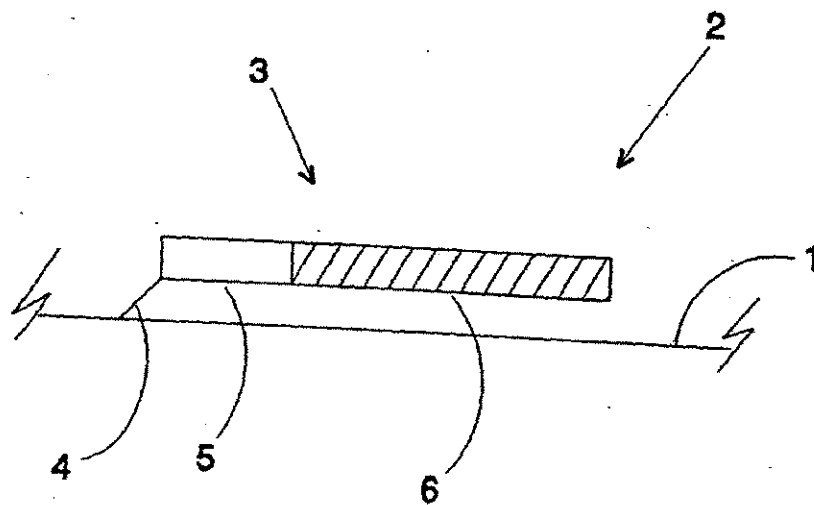


Figure 1a

IAFP00013512

WO 93/17126

2/14

PCT/US93/01552

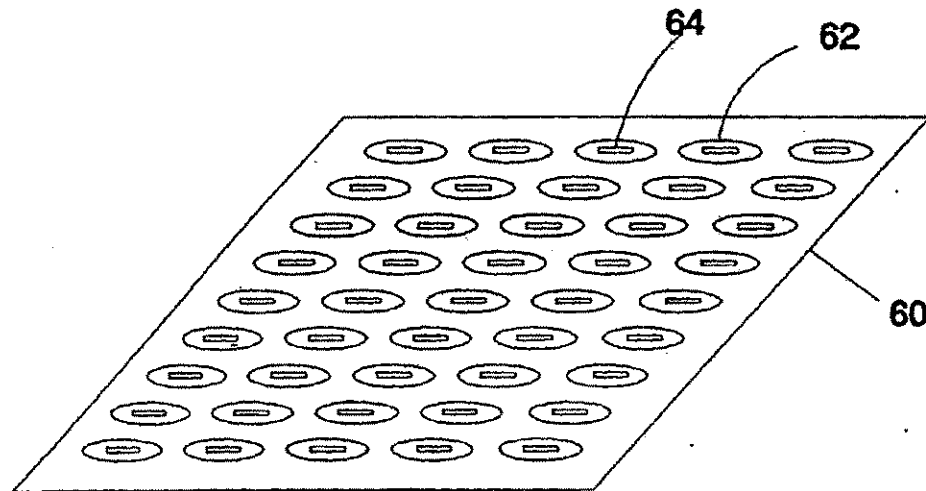


Figure 2 - Sectioned Array

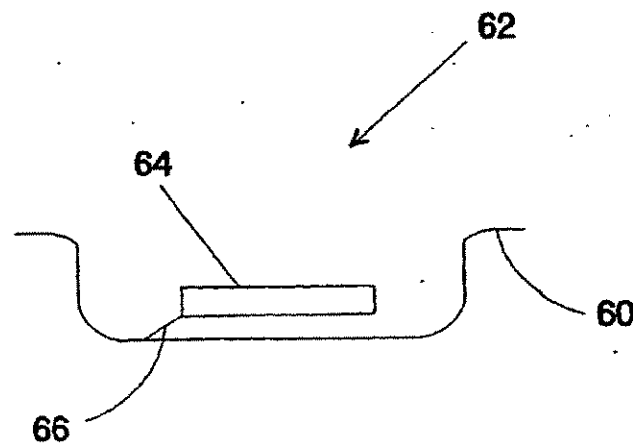


Figure 2a

IAFP00013513

WO 93/17126

3/14

PCT/US93/01552

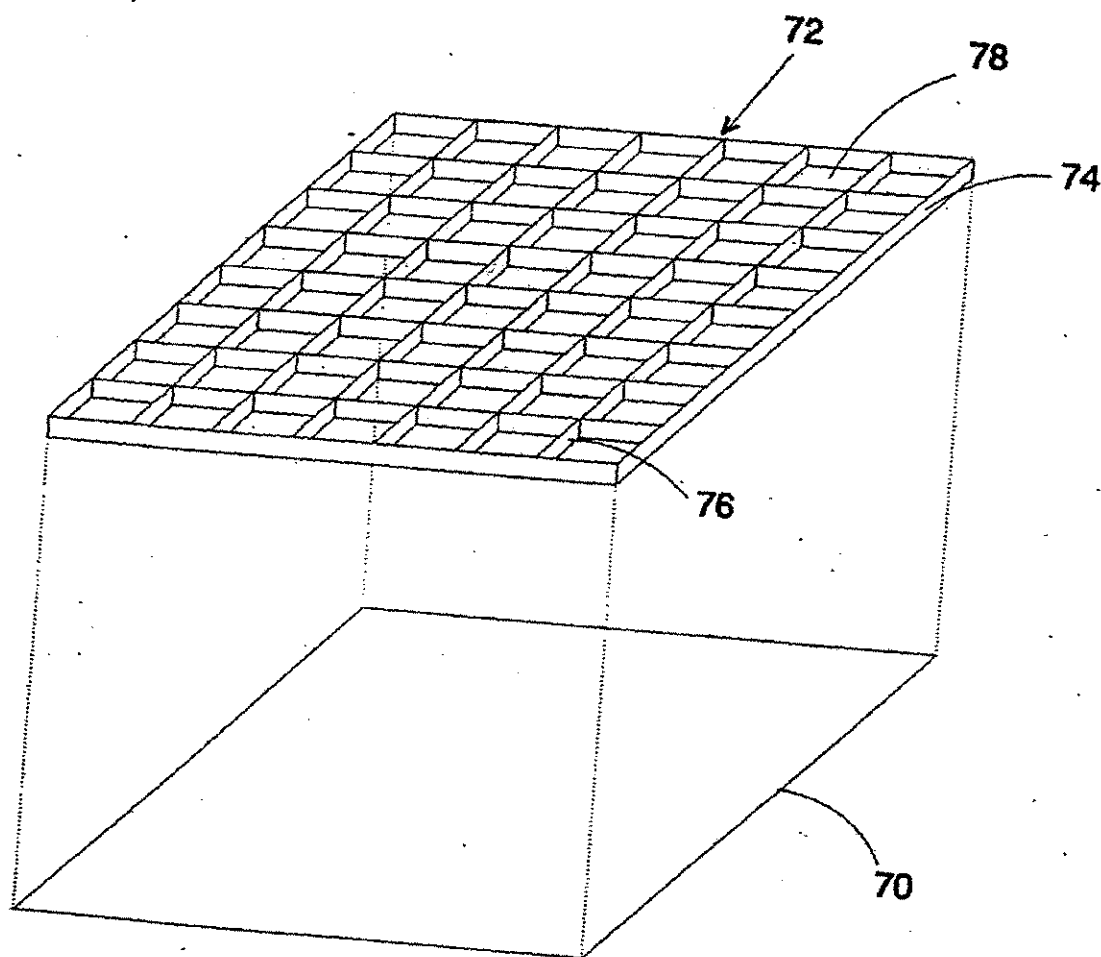


Figure 3

IAFP00013514

WO 93/17126

PCT/US93/01552

4/14

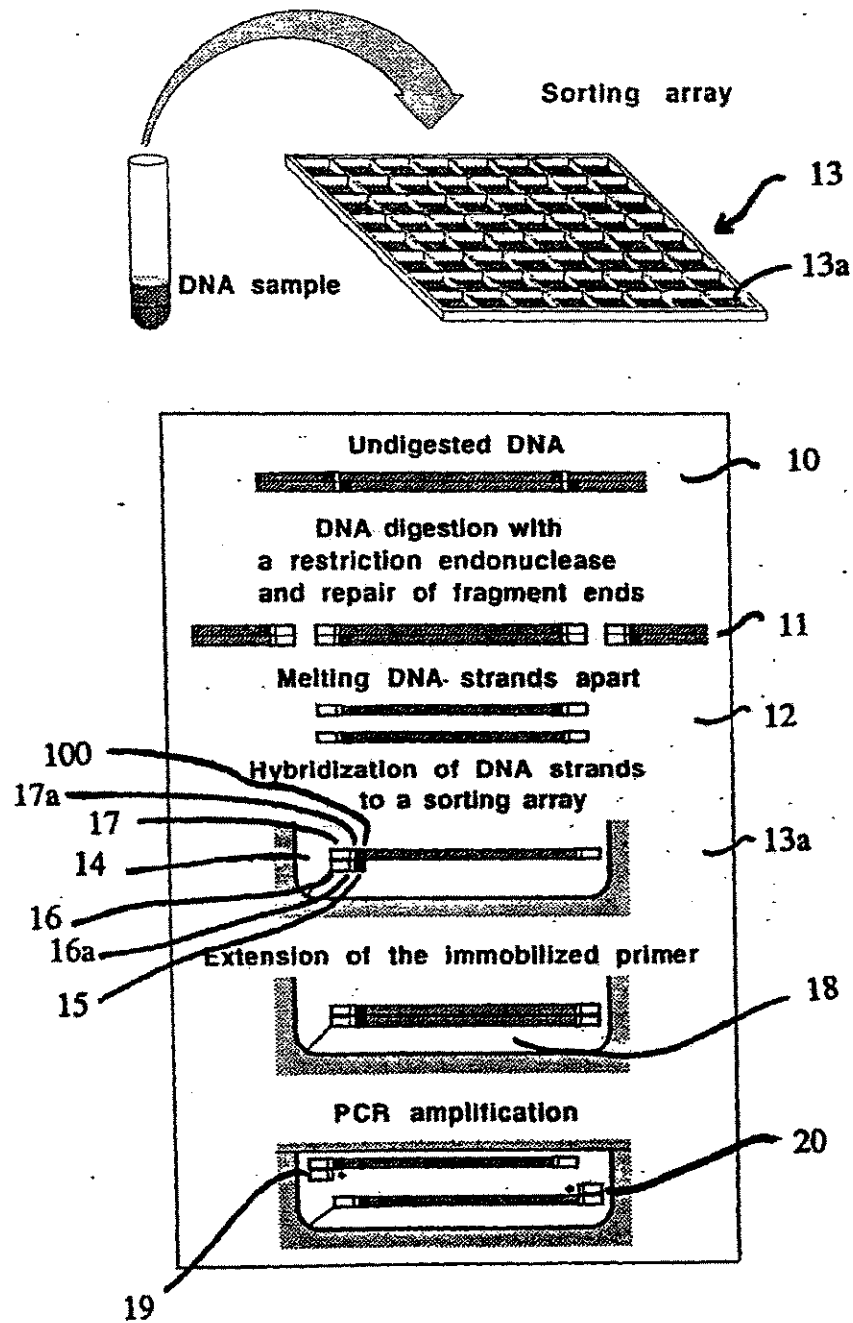


Figure 4

WO 93/17126

PCT/US93/01552

5/14

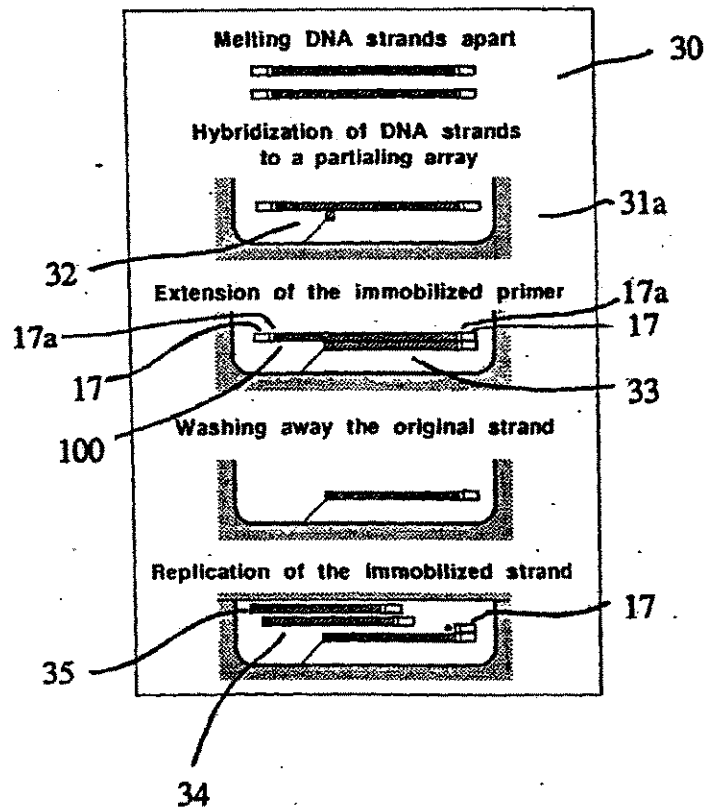
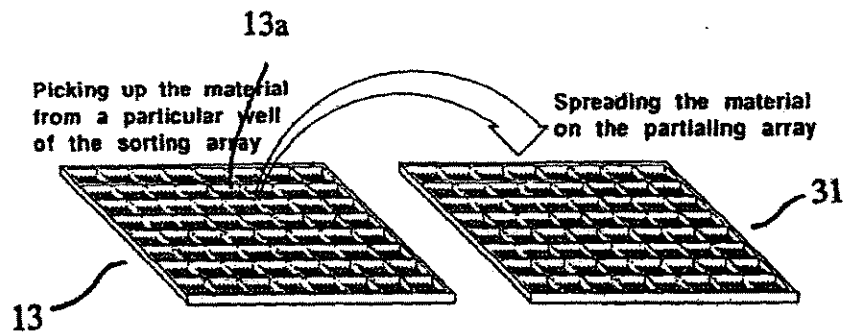


Figure 5

WO 93/17126

PCT/US93/01552

6/14

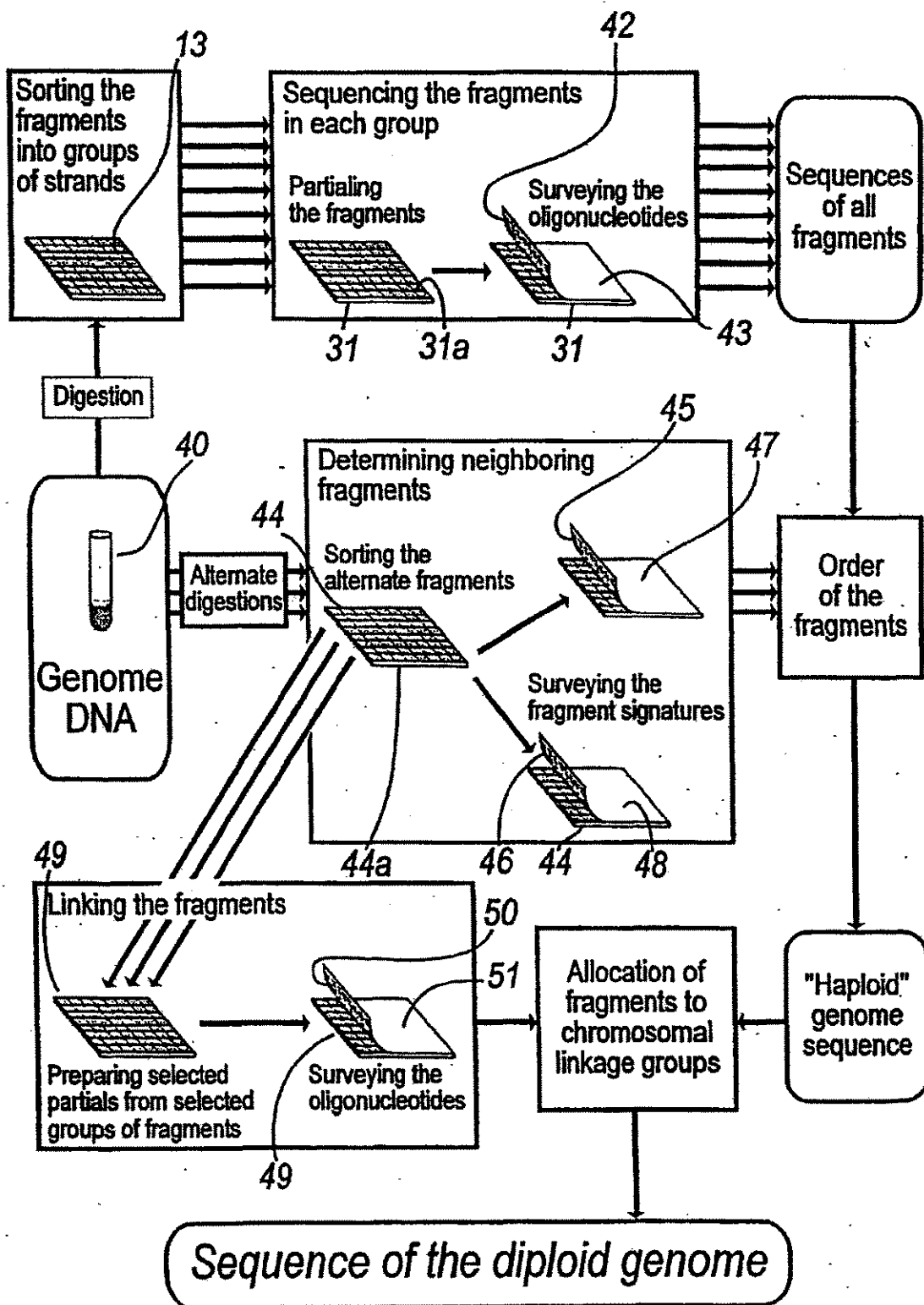


Figure 6

SUBSTITUTE SHEET

IAFP00013517

WO 93/17126

7/14

PCT/US93/01552

A single survey array

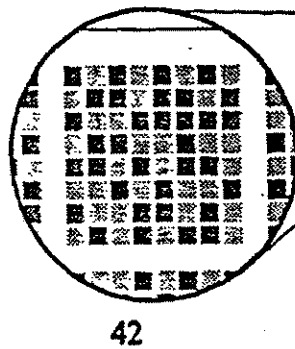


Figure 7a

Surveying oligonucleotide  
content of the partials

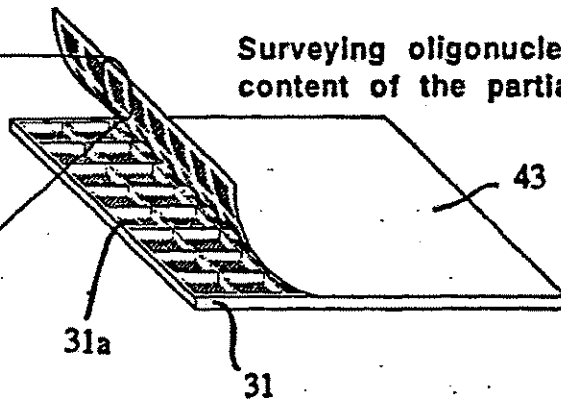


Figure 7b

**PCT/US93/01552**

8/14

## Strands in the mixture

CATGGTACCTTGGTAA

**ATGGTCCTTGGTACCTA**

Fig. 8a

## Indexed address sets

**Fig. 3b**

## Indexed address sets

**Fig. 3b**

[illegible]

WO 93/17126

PCT/US93/01552

9/14

## Unindexed address sets

8c

ACC	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC	TGG	TTG
ATG	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC	TGG	TTG
CAT	ACC	ATG	CAT	CCT		CTT	GGT	GTA		TAA	TAC		TGG	TTG
CCT	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC	TGG	TTG
CTA	ACC	ATG		CCT	CTA	CTT	GGT	GTA	GTC		TAC	TCC	TGG	TTG
CTT	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC	TGG	TTG
GGT	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC	TGG	TTG
GTA	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC	TGG	TTG
GTC	ACC	ATG		CCT	CTA	CTT	GGT	GTA	GTC		TAC	TCC	TGG	TTG
TAA	ACC	ATG	CAT	CCT		CTT	GGT	GTA		TAA	TAC		TGG	TTG
TAC	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC	TGG	TTG
TCC	ACC	ATG		CCT	CTA	CTT	GGT	GTA	GTC		TAC	TCC	TGG	TTG
TGG	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC	TGG	TTG
TTG	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC	TGG	TTG

## Grouped address sets

8d

CAT	ACC	ATG	CAT	CCT		CTT	GGT	GTA		TAA	TAC		TGG	TTG
TAA	ACC	ATG	CAT	CCT		CTT	GGT	GTA		TAA	TAC		TGG	TTG
CTA	ACC	ATG		CCT	CTA	CTT	GGT	GTA	GTC		TAC	TCC	TGG	TTG
GTC	ACC	ATG		CCT	CTA	CTT	GGT	GTA	GTC		TAC	TCC	TGG	TTG
TCC	ACC	ATG		CCT	CTA	CTT	GGT	GTA	GTC		TAC	TCC	TGG	TTG
ACC	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC	TGG	TTG
ATG	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC	TGG	TTG
CCT	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC	TGG	TTG
CTT	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC	TGG	TTG
GGT	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC	TGG	TTG
GTA	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC	TGG	TTG
TAC	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC	TGG	TTG
TGG	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC	TGG	TTG
TTG	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC	TGG	TTG

I

II

III

WO 93/17126

PCT/US93/01552

10/14

## Identified strand sets

A: ACC ATG CAT CCT CTT GGT GTA TAA TAC TGG TTG

CAT	ACC	ATG	CAT	CCT	CTT	GGT	GTA	TAA	TAC	TGG	TTG
TAA	ACC	ATG	CAT	CCT	CTT	GGT	GTA	TAA	TAC	TGG	TTG
ACC	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC
ATG	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC
CCT	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC
CTT	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC
GGT	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC
GTA	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC
TAC	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC
TGG	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC
TTG	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC

I

III

Figure 9a

B: ACC ATG CCT CTA CTT GGT GTA GTC TAC TCC TGG TTG

CTA	ACC	ATG	CCT	CTA	CTT	GGT	GTA	GTC	TAC	TCC	TGG	TTG
GTC	ACC	ATG	CCT	CTA	CTT	GGT	GTA	GTC	TAC	TCC	TGG	TTG
TCC	ACC	ATG	CCT	CTA	CTT	GGT	GTA	GTC	TAC	TCC	TGG	TTG
ACC	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC
ATG	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC
CCT	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC
CTT	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC
GGT	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC
GTA	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC
TAC	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC
TGG	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC
TTG	ACC	ATG	CAT	CCT	CTA	CTT	GGT	GTA	GTC	TAA	TAC	TCC

II

III

Figure 9b

SUBSTITUTE SHEET

**WO 93/17126**

11/14

PCT/US93/01552

## Strand set A

ACC ATG CAT CCT CTT GGT GTA TAA TAC TGG TTG

Fig. 10a

## Assembling oligonucleotides into blocks

CAT ATG TAA TAC ACC CCT CTT TTG TGG GGT GTA

CATG TAA TACCTG TGCTA

Fig. 10b

## Converting filtered address sets into block sets

[illegible]

WO 93/17126

PCT/US93/01552

12/14

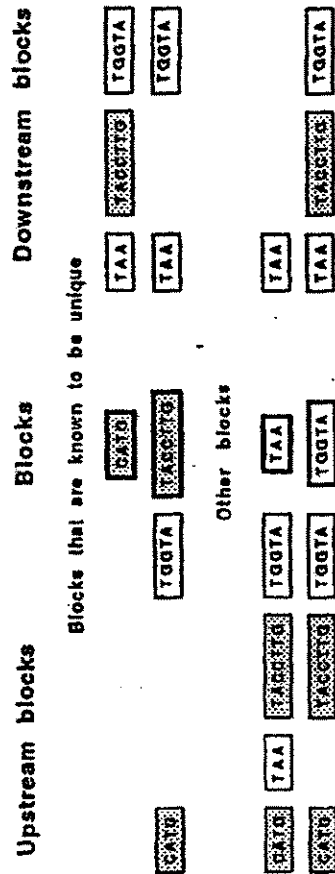


Fig. 10d

## Ordering the blocks

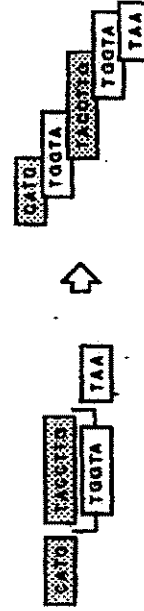


Fig. 10e

**Sequence: CATGGTACCTTGGTAA**

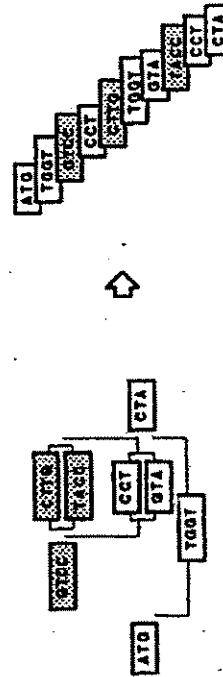
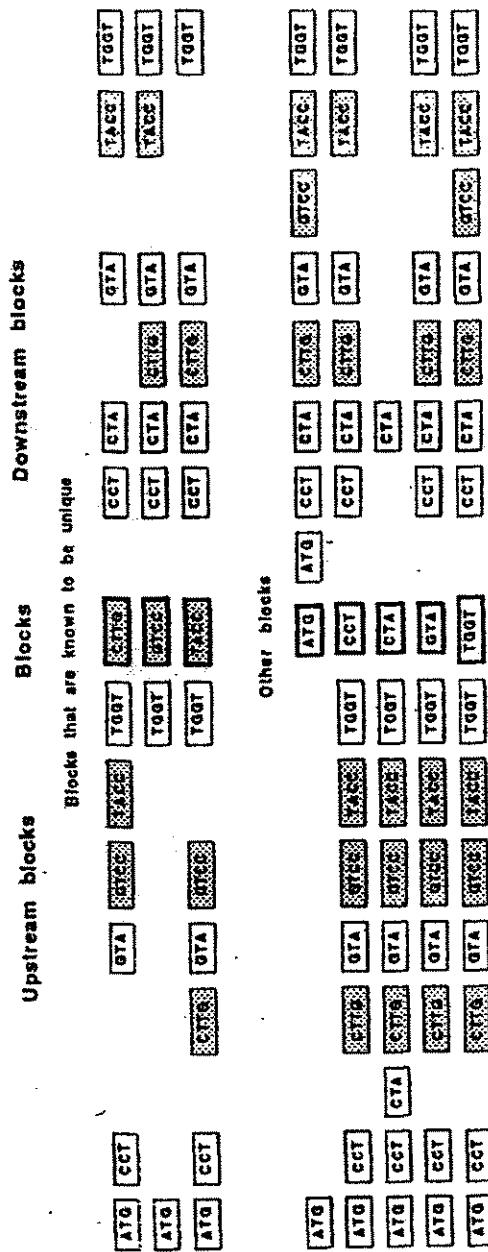
Fig. 10f



**WO 93/17126**

**PCT/US93/01552**

14/14



**Sequence: ATGGTCCTTGGTACCTA**

Fig. 11f

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US93/01552

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(S) : C12Q 1/68; C12P 19/34

US CL : 435/6, 91

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 91

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG (One search-BIOCHEM), STN, APS +

Search terms: binary oligonucleotide array; sequencing and oligonucleotides and DNA; oligonucleotide probe

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<u>Journal of Theoretical Biology</u> , Volume 135, issued 1988, Bains, <i>et alii</i> , "A Novel Method for Nucleic Acid Sequence Determination", pages 303-307, see entire document.	1-197
Y	<u>Journal of Biomolecular Structure &amp; Dynamics</u> , Volume 7, Issue Number 1, issued 1989, Pevzner, "1-Tuple DNA Sequencing: Computer Analysis", pages 063-069, see entire document.	1-197
Y	<u>Science</u> , Volume 253, issued 27 September 1991, Barinaga, "Will 'DNA Chip' Speed Genome Initiative?", page 1489.	1-197

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be part of particular relevance	X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
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"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

01 APRIL 1993

Date of mailing of the international search report

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IAFP00013526

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US93/01552

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Lysov, <i>et alii</i> , "A New Method for Determining the DNA Nucleotide Sequence by Hybridization with Oligonucleotides", published December 1988 by Doklady Akademii Nauk SSSR (Moscow), pp. 1508-1511, see entire document.	1-197
Y	Drmanac, <i>et alii</i> , "Partial Sequencing by Oligo-Hybridization: Concept and Applications in Genome Analysis", <u>The First International Conference on Electrophoresis, Supercomputing and the Human Genome</u> , published April 1990 by World Scientific (NJ), see entire document.	1-197
Y	<u>DNA and Cell Biology</u> , Volume 9, Number 7, issued 1990, Drmanac, <i>et alii</i> , "Laboratory Methods: Reliable Hybridization of Oligonucleotides as Short as Six Nucleotides", pages 527-534, see entire document.	1-197
Y	<u>FEBS Letters</u> , Volume 256, Number 1, 2, issued October 1989, Khrapko, <i>et alii</i> , "An oligonucleotide hybridization approach to DNA sequencing", pages 118-122, see entire document.	1-197
Y	WO, A, 89/10977 (SOUTHERN) 16 NOVEMBER 1989, see entire document.	1-197
Y	US, A, 5,002,867 (MACEVICZ) 26 MARCH 1991, see entire document.	1-197
Y	EP, A, 0,392,546 (DRMANAC ET AL.) 17 OCTOBER 1990, see entire document.	1-197
Y	DNA Sequence - J. DNA Sequencing and Mapping, Vol. 1, issued 1991, Khrapko et al., "A Method for DNA Sequencing by Hybridization with Oligonucleotide Matrix", pages 375-388, see entire document.	1-197

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